Growth Kinetics of *Listeria monocytogenes* in Broth and Beef Frankfurters—Determination of Lag Phase Duration and Exponential Growth Rate under Isothermal Conditions

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ABSTRACT: The objective of this study was to develop a new kinetic model to describe the isothermal growth of microorganisms. The new model was tested with *Listeria monocytogenes* in tryptic soy broth and frankfurters, and compared with 2 commonly used models—Baranyi and modified Gompertz models. Bias factor (BF), accuracy factor (AF), and root mean square errors (RMSE) were used to evaluate the 3 models. Either in broth or in frankfurter samples, there were no significant differences in BF (approximately 1.0) and AF (1.02 to 1.04) among the 3 models. In broth, the mean RMSE of the new model was very close to that of the Baranyi model, but significantly lower than that of the modified Gompertz model. However, in frankfurters, there were no significant differences in the mean RMSE values among the 3 models. These results suggest that these models are equally capable of describing isothermal bacterial growth curves. Almost identical to the Baranyi model in the exponential and stationary phases, the new model has a more identifiable lag phase and also suggests that the bacteria population would increase exponentially until the population approaches to within 1 to 2 logs from the stationary phase. In general, there is no significant difference in the means of the lag phase duration and specific growth rate between the new and Baranyi models, but both are significantly lower than those determined from the modified Gompertz models. The model developed in this study is directly derived from the isothermal growth characteristics and is more accurate in describing the kinetics of bacterial growth in foods.

Keywords: growth kinetics, modeling, predictive microbiology

Introduction

The growth of microorganisms in foods is usually a 3-stage process, generally categorized as the lag, exponential, and stationary phases. Under any conditions suitable for a microorganism to grow, the lag phase can be observed where there is no apparent change in the microbial population. Microbial growth usually exhibits a lag phase when there is a change in the environmental conditions or a bacterial culture is introduced into a new environment. The lag phase allows the bacteria to adjust to the new growth environment before the exponential phase starts, during which bacterial cells actively divide and multiply and a logarithmic increase in bacterial population occurs. At the stationary phase, the population of bacteria reaches the maximum density and equilibrium in the bacterial growth is established. At this final stage of bacterial growth, there is also no apparent change in the cell population. Accurate categorization and quantification of each of the bacterial growth phases are essential to the determination of the length of the lag phase and the rate of bacterial growth, which is critical to the evaluation of microbial safety of foods and establishment of shelf life.

Under isothermal conditions, the bacterial growth curves are usually sigmoid-shaped and can be described by several mathematical models. One of the more frequently used empirical models is modified from the Gompertz model (Gibson and others 1987), expressed as

$$\log(C) = A + (B - A)\exp\{-\exp[-\mu_G(t - M)]\} \quad (1)$$

From the modified Gompertz model, the duration of the lag phase (λ) and the specific growth rate (K) of a growth curve under an isothermal condition can be derived:

$$K = \frac{(B - A)\,\mu_G}{e} \quad (2)$$

and

$$\lambda = M - \frac{1}{\mu_G} \quad (3)$$

In Eq. 1 to 3, $\log(C)$ is the logarithm (base 10) of bacterial counts (CFU/g); A and B are the logarithm of the initial cell concentration and the stationary phase maximum cell concentration [log(CFU/g)]; μ_G is the relative growth rate at $t = M$ (per hour), which is the inflection point of the curve; λ is the duration of the lag phase; and K is the specific growth rate [log(CFU/g)/h] of an isothermal growth curve (Gibson and others 1987).

Since the modified Gompertz model is basically an empirical model that can be used to fit the sigmoid trend of an isothermal growth curve, it may not have any direct physical or biological meaning. Therefore, another more kinetically based growth model (Baranyi model), has been proposed and evolved through the years into the current form (Baranyi and others 1993, 1995):

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$$y(t) = y_0 + \mu_{max} A(t) - \ln\left\{1 + \frac{\exp[\mu_{max} A(t)] - 1}{\exp(y_{max} - y_0)}\right\} \quad (4)$$

where, $y(t)$ is the natural logarithm of bacterial counts, or $\ln(C)$; y_0 and y_{max} are the natural logarithm of the initial and the stationary phase maximum bacterial counts; μ_{max} is maximum growth rate (based on natural logarithm); and $A(t)$ is defined by

$$A(t) = t + \frac{1}{\nu}\ln[\exp(-\nu t) + \exp(-h_0) - \exp(-\nu t - h_0)] \quad (5)$$

According to Baranyi and Roberts (1994), the lag phase duration is affected by ν. If it is assumed that the critical substances are formed at the same rate as the bacteria grow, ν is equal to μ_{max}. According to Baranyi and Roberts (1994) and Baranyi and others (1995), the lag phase duration can be calculated from

$$\lambda = \frac{h_0}{\mu_{max}} \quad (6)$$

Similar to the modified Gompertz curve, λ is actually the value of time at the intercepting point between $y = y_0$ and the line tangential to the inflexion point of the growth curve in the exponential phase for the Baranyi model. The parameter h_0 is the distance between y_0 and the point where the line tangential to the inflexion point of the growth curve intercepts the y-axis. The specific growth rate K, which is based on the logarithm of base 10, is equal to $\mu_{max}/\ln(10)$, or $\mu_{max}/2.303$. For the sake of clarity in this article, K is termed as the specific growth rate and μ_{max} as the maximum growth rate, although the physical and biological meanings of the 2 terms are the same.

The development of the Baranyi model relies on the concept of physiological state of cells, which is basically a virtual parameter and is extremely difficult to be observed, validated, and quantified experimentally. The parameter h_0 defines the lag phase in the Baranyi model (Eq. 6). According to Baranyi's hypothesis, a lag phase is usually observed and the transition from the lag phase to the exponential phases depends upon the formation of "critical substances" when bacterial cells are transferred to a new growth medium and exposed to a new environment (Baranyi and Roberts 1994; Baranyi and others 1995). This assumption sometimes is contradictory to experimental observations. When a population of healthy, fully developed, actively dividing cells is inoculated to a different environment containing all necessary components for healthy cells to grow, a lag phase may still exist, even though all the "critical substances" are available extracellularly and intracellularly.

The objective of this study was to develop a new intuitive and more biologically based kinetic model to describe the growth of microorganisms in foods and compare it with the Baranyi and modified Gompertz models. The aim was to develop a biological growth model that was not limited by any previous growth conditions and history, and was completely based on the current growth environment and the trend of the growth curve.

Materials and Methods

Model development—full model

Without the lag and stationary phases, the multiplication of a microorganism in a food system usually follows 1st-order growth kinetics, which can be described as

$$\frac{dC}{dt} = \mu_{max} C \quad (7)$$

If the bacteria are allowed to grow strictly following the 1st-order kinetics (Eq. 7), the number of bacteria increases exponentially. Since in any growth environment the bacterial growth is limited by a maximum capacity, or C_{max}, which is the maximum cell density and usually represents the cell concentration in the stationary phase, the process of bacterial growth is smoothly modeled between the exponential and stationary phases by

$$\frac{dC}{dt} = kC(C_{max} - C) \quad (8)$$

where $kC_{max} = \mu_{max}$ when C is $\ll C_{max}$.

The equation expressed in Eq. 8 depicts a process that the bacteria start to multiply immediately after inoculation, and therefore it is not suitable for describing the entire growth curve if there is a lag phase. For a process containing all the phases, including lag, exponential, and stationary phases, the growth process can be expressed as

$$\begin{aligned} \frac{dC}{dt} &= 0, \quad \text{if } t \le \lambda, \\ \frac{dC}{dt} &= kC(C_{max} - C), \quad \text{if } t > \lambda \end{aligned} \quad (9)$$

Equation 9 is a general model that describes the entire process of bacterial growth. During the lag phase, there is no change in the number of cells, and therefore $dC/dt = 0$. After the lag phase, the bacterial growth begins and the exponential phase starts following Eq. 8.

Although Eq. 9 can be used to describe the entire growth process, it is a discontinuous model that requires 2 separate equations. A single equation can be developed using a unit step function to combine the 2 equations:

$$\frac{dC}{dt} = U(t - \lambda) \times kC(C_{max} - C) \quad (10)$$

The unit function used in Eq. 10 has a unique mathematical property. If $t \le \lambda$ (within the lag phase), $U(t-\lambda) = 0$. At any moment when $t > \lambda$, $U(t-\lambda) = 1$. With $U(t-\lambda) = 0$, there is no growth. With $U(t-\lambda) = 1$, the bacterial growth follows Eq. 8. The unit step function used in Eq. 10 joins 2 separate expressions in Eq. 9 into a single equation. However, the unit step function is also a discrete function, and therefore not continuous. To make Eq. 10 continuous, a smooth transitional unit function is needed. Transitional functions, $f(t)$, can be used to allow a smooth transition from the lag phase to the exponential phase in the model:

$$\frac{dC}{dt} = kC(C_{max} - C) \times f(t) \quad (11)$$

where $f(t)$ is a transitional function that defines the duration of the lag phase of a growth curve, and one such function is

$$f(t) = \frac{1}{1 + \exp[-\alpha(t - \lambda)]} \quad (12)$$

This transitional function $f(t)$ has the mathematical property that if $t \ll \lambda$, $f(t) = 0$; and if $t >> \lambda$, $f(t) = 1$. When t is in the vicinity of λ, $f(t)$ gradually changes from 0 to 1. The coefficient α defines the sharpness of the transition in the growth curve. With $f(t)$ available, the entire growth process can be described as

$$\frac{dC}{dt} = \frac{kC(C_{max} - C)}{1 + \exp[-\alpha(t - \lambda)]} \quad (13)$$

The differential growth equation expressed in Eq. 13 is a continuous one that can be solved analytically by separation of variables. The general growth model can be expressed as:

$$y(t) = y_0 + y_{max} - \ln\{\exp(y_0) + [\exp(y_{max}) - \exp(y_0)] \times \exp[-k \times \exp(y_{max}) B(t)]\},$$
$$\text{where } B(t) = t + \frac{1}{\alpha} \ln \frac{1 + \exp[-\alpha(t-\lambda)]}{1 + \exp(\alpha\lambda)} \quad (14)$$

With the selection of a sufficiently large α, Eq. 14 is the full continuous growth model that covers the entire range from the lag phase, through the exponential phase, and finally to the stationary phase. In this model, the duration of the lag phase (λ) is clearly identified. The variables, y_0, y_{max}, and $y(t)$, are identical to those used in the Baranyi model. The variable k is the rate constant for the exponential phase in Eq. 8. It is not the specific growth rate, and has the unit of $(CFU/g{\cdot}time)^{-1}$.

The unit transitional function equals 1 after the lag phase, and the rate of growth is not affected by it for $t > \lambda$. After the lag phase, the bacterial growth would follow the relationship outlined in Eq. 9. Therefore, the maximum rate of bacterial growth can be obtained from this equation. The real-time growth rate based on the natural logarithm of the bacterial counts can be determined from:

$$\mu = \frac{dy}{dt} = \frac{d[\ln(C)]}{dt} = \frac{1}{C}\frac{dC}{dt} = k(C_{max} - C) \quad (15)$$

The maximum growth occurs when $C = C_0$ when the bacterial count is minimal. Therefore, the maximum growth rate can be calculated from

$$\mu_{max} = k(C_{max} - C_0) \quad (16)$$

Special case—reduced model

Occasionally, a complete growth curve containing all 3 phases may not be available to develop a full model. The growth curve may only contain the lag and exponential phases. In such a case, the growth curve from the lag to the exponential phases can be described by

$$\frac{dC}{dt} = \frac{\mu C}{1 + \exp[-\alpha(t-\lambda)]} \quad (17)$$

The above-mentioned differential equation also can be solved by separation of valuables, resulting in

$$y(t) = y_0 + \mu \left\{ t + \frac{1}{\alpha} \ln \frac{1 + \exp[-\alpha(t-\lambda)]}{1 + \exp(\alpha\lambda)} \right\} \quad (18)$$

Preparation of bacteria

Four strains of *Listeria monocytogenes* (H7763, H7776, H7778, and 46877) were obtained from the culture collection of USDA Agricultural Research Service (ARS) Eastern Regional Center (ERRC) located at Wyndmoor, Pa., U.S.A. The stock cultures were regularly propagated and maintained on tryptic soy agar (TSA, BD/Difco Laboratories, Sparks, Md., U.S.A.) plates and stored at 4 °C.

One day before the experiment, a loopful of each strain was individually transferred to 10 mL brain heart infusion broth (BHI broth, BD/Difco Laboratories) and held at 37 °C on an orbital shaker (approximately 100 rpm) for approximately 22 to 24 h. The bacteria cultures were harvested by centrifugation (2400 × *g*, 15 min, 4 °C), washed once with 10 mL of 0.1% peptone water (PW, BD/Difco Laboratories), recentrifuged, and resuspended in 1 mL PW. A 4-mL cocktail was formed by combining and mixing the 1-mL suspensions of each strain. The cocktail contained approximately $10^{9.5}$ CFU/mL of *L. monocytogenes* cells. Serial dilutions were made from the *Listeria* cocktail for growth studies. A fresh cocktail was prepared for each experiment.

Isothermal growth in broth

Tryptic soy broth (TSB, BD/Difco Laboratories) was used to study isothermal growth in broth. Four 500-mL Erlenmeyer flasks, each containing 200-mL sterile TSB, were inoculated with *L. monocytogenes*. Based on a preliminary study, a small volume of serially diluted cocktail solutions was added to TSB. After inoculation, the initial concentration of the bacteria in TSB was approximately 1, 2, 3, and 4 log(CFU/mL), labeled as G1, G2, G3, and G4, respectively. A sterile magnetic stirring bar was added to each flask containing inoculated TSB, which was placed in a temperature-controlled orbital shaker (Labline Environmental Shaker, Model 4628, Barnstead/Thermolyne, Melrose Park, Ill., U.S.A.) operated at 120 rpm. The temperature of the orbital shaker was controlled at 37 °C.

At predetermined time intervals, the flasks were taken out of the orbital shaker and placed on magnetic stirrers. The magnetic bars inside each flask were rotated at high speed to mix the contents in the broth. After being mixed, the samples were withdrawn and plated onto tryptic soy agar (TSA, BD/Difco Laboratories) plates directly or after serial dilution with PW. The original inoculum was also plated to calculate the initial inoculum level in TSB. The TSA plates were held in an incubator for 48 h at 37 °C. The bacterial colonies were counted and converted to the logarithm of the natural base or base 10, and recorded as ln(CFU/mL) or log(CFU/mL). Each experiment was conducted in triplicate.

Isothermal growth in beef frankfurters

Growth studies were also conducted in beef frankfurters, which were obtained from a local manufacturer. The frankfurters, containing no lactates or diacetates as antimicrobial agents, were divided into 1 ± 0.05 g portions and placed in filter bags (Whirl-Pak®, 7 oz., 95 × 180 × 0.08 mm, NASCO, Fort Atkinson, Wis., U.S.A.). According to the manufacturer, the water vapor and oxygen transmission rates of the filter bags were 7.8 g/m^2/24 h and 3100 mL/m^2/24 h, respectively. The beef frankfurter sample in each bag was flattened with a round bottle and 0.1-mL diluted cocktail of *L. monocytogenes* was added. The filter bags were vacuum-sealed at 1200 Pa (12 mBar). The initial inoculum level was approximately 3 log(CFU/g) in beef frankfurters.

Inoculated samples were placed in incubators maintained at 15, 25, 30, 37, or 40 °C. At time intervals determined by the incubation temperature, samples were removed from incubators, added with 5 mL PW, and mixed for 3 min at the maximum speed in a stomacher (Model BagMixer® 100W, Interscience Co., France). A small volume of the liquid portion of the stomached samples was withdrawn and plated, either directly or after serial dilution, onto freshly prepared PALCAM *Listeria* selective agar (BD/Difco Laboratories) plates (Van Netten and others 1989). The PALCAM plates were incubated at 37 °C for approximately 48 h. After incubation, typical *Listeria* colonies were counted, averaged, and converted to the logarithm (natural base or base 10) of CFU per gram of frankfurters. Three independent growth experiments were conducted for each incubating temperature.

Mathematical modeling

The logarithms of bacterial counts were fitted to 3 growth models—the new model (full or reduced), Baranyi model, and modified Gompertz model. For the new and Baranyi models, the natural logarithms were used. For the modified Gompertz model, the base 10 logarithms were used. A nonlinear regression procedure based on Gauss–Newton method in Windows-based SAS version 9.1.3 (SAS Inst. Inc., Cary, N.C., U.S.A.) was used to fit the growth data to the models. After the statistical analysis was completed, a pseudo-R^2 value was calculated for each model using the following equation:

$$R^2 = 1 - \frac{\sum_1^n \left(Y_i - \hat{Y}_i\right)^2}{\sum_1^n \left(Y_i - \bar{Y}\right)^2} \tag{19}$$

where Y_i are the logarithms (base e or 10) of bacterial counts, $\hat{Y}_i$ are the logarithms of bacterial counts estimated by a model, and $\bar{Y}$ is the average of the logarithms of bacterial counts observed experimentally.

Evaluation of isothermal models

The bias factor and accuracy factors proposed by Ross (1996) were used to evaluate the performance of each growth model. The bias factor (BF) was calculated from

$$\mathrm{BF} = 10^{\frac{1}{n}\sum \log_{10}\left(\frac{\text{predicted}}{\text{observed}}\right)} \tag{20}$$

The accuracy factor (AF) was determined from

$$\mathrm{AF} = 10^{\frac{1}{n}\sum \left|\log_{10}\left(\frac{\text{predicted}}{\text{observed}}\right)\right|} \tag{21}$$

Another parameter, root mean square error (RMSE), an estimate of the standard error of a model, was also calculated for each model:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum \left(Y_i - \hat{Y}_i\right)^2} \tag{22}$$

For the calculation of BF, AF, and RMSE, all bacterial counts were converted to base 10 logarithms. Analysis of variance (ANOVA) was conducted to compare of the mean of K, λ, BF, AF, and RMSE among different models. The Tukey's Studentized range (HSD) test procedure was used to group the means of K, λ, BF, AF, and RMSE. The statistical analyses were conducted using Windows-based SAS version 9.1.3 (SAS Inst. Inc.).

Results and Discussion

Modeling of isothermal growth in broth—full model

With the growth data from the lag phase to the stationary phase, all 3 models can be used to fit the growth curves (Figure 1). Regardless of the level of initial inoculum in TSB, all growth curves start from the initial concentrations, smoothly change to the exponential phase, and stabilize at the stationary phase. The lag phase of each growth curve is clearly defined by the new model and is visibly distinguishable from the exponential phase (Figure 1). For *L. monocytogenes* in TSB at 37 °C, the lag phases can be directly identified from the growth curves fitted to the new model. For the modified Gompertz and Baranyi models, however, the lag phases are not clearly distinguishable from the exponential phases, and the calculated bacterial population starts to increase immediately for these 2 models (Figure 1). The lag phases must be calculated from the mathematical equations using either Eq. 3 or 6, which is actually the x-value of the intercepting point between $y = y_0$ [or $\log(C) = \log(C_0)$] and the line tangential to the inflection point (at which the relative growth rate is highest) of the growth curve, for both Baranyi and modified Gompertz models. The duration of the lag phases, obtained either directly from the model or calculated from the modified Gompertz model or the Baranyi model, was not significantly affected by the initial inoculum level ($P = 0.5$). There was no significant difference in the mean of the lag phases determined from the new and Baranyi models (Table 1). However, the mean lag phase duration calculated from the modified Gompertz model was significantly higher than those determined from the new and Baranyi models. The mean lag phase duration calculated from the Gompertz model was 71% and 61% higher than that of the Baranyi model and the new model, respectively, for *L. monocytogenes* in TSB held at 37 °C (Table 1).

Regardless of the level of initial inoculum, the bacterial growth moved through the exponential phase to the stationary phase. Again, the stationary phase bacterial concentration was not

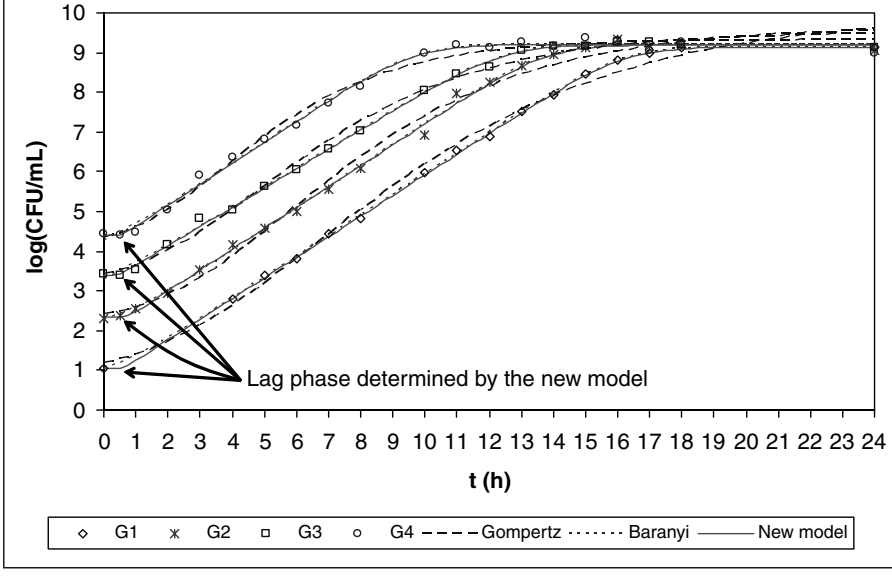

Figure 1—The growth of *L. monocytogenes* in TSB modeled by the modified Gompertz, Baranyi, and new (full) models. G1 to G4 represent different initial concentrations.

dependent on the initial inoculum concentrations ($P = 0.148$), but was dependent on the model used to fit the growth data. The mean maximum cell concentration determined from the new model was identical to that from the Baranyi model (Table 1). However, the mean maximum cell concentration determined from the modified Gompertz [9.68 log(CFU/mL)] was significantly higher than the maximum cell concentration determined from the new and Baranyi models [9.13 log(CFU/mL); Table 1].

In the exponential phase, bacteria multiply exponentially, and the number or concentration of bacteria doubles periodically, following the 1st-order kinetics. A plot of the logarithm of the bacterial counts against the growth time should be a linear curve. The linear characteristics of bacterial growth can be represented by both the new and the Baranyi models, as the curves in the exponential phase sections appear linear in Figure 1. The curves in the exponential phase sections are almost identical to each other for the new and Baranyi models. The curves in the exponential phase section for the modified Gompertz are apparently not linear for a long period. The means of the specific growth rates determined from the new and Baranyi models were similar (Table 1). However, the mean specific growth rate determined from the modified Gompertz model is higher than the mean specific growth rates determined from the new and Baranyi models (Table 1). Again, the initial inoculum level did not affect the specific growth rate ($P = 0.541$). The curve sections corresponding to the exponential phase of the new model were almost parallel (Figure 1).

The average rate constant k in Eq. 8 was 8.56×10^{-10}/(CFU/mL/h)] with a standard deviation of 1.62×10^{-10} [/(CFU/mL/h)]. At any given time t, the growth rate (μ), or the increase in the natural logarithm counts of bacteria per unit time, is determined by k and the difference in the maximum cell concentration and the cell concentration at time t (Eq. 15):

$$\mu = \frac{dy}{dt} = \frac{d[\ln(C)]}{dt} = \frac{1}{C}\frac{dC}{dt} = k(C_{\max} - C) \qquad (23)$$

Since $C_{\max}$ is in the range of 1.0×10^9, and if the cell concentration in the exponential phase is small (1 to 10^7), then the difference between $C_{\max}$ and C is almost negligible and the population of the bacteria will increase at the maximum bacterial growth rate ($\mu_{\max}$) before the cell concentration approaches within to 1 to 2 logs of the maximum cell concentration (Figure 2). Therefore, after exiting the lag phase, the bacteria would grow in a linear manner until the cell concentration is near the maximum cell concentration. The linearity of the new growth model fits well with bacterial growth in the exponential phase, and is suitable for describing isothermal bacterial growth. According to this model, at low levels of initial inoculum [$<10^7$ log(CFU/mL)], the inoculum concentration does not affect the rate of bacterial growth in broth, which agrees very well with the experimental observations obtained in this study.

Table 1—Mean lag phase duration (λ), specific growth rate (*K*), and stationary phase concentration determined from 3 models for *L. monocytogenes* in TSB.

Model	λ (h)	*K* [log(CFU/mL)/h]	Stationary phase concentration [log(CFU/mL]
Gompertz	$1.40^{a,A}$ (0.55)[B]	0.605^{a} (0.05)	9.68^{a} (0.39)
Baranyi	0.82^{b} (0.35)	0.505^{b} (0.04)	9.13^{b} (0.10)
New model	0.87^{b} (0.29)	0.501^{b} (0.04)	9.13^{b} (0.09)

[A]The mean value of a parameter (λ, *K*, or stationary phase cell concentration). Superscripts with the same letter in the same column were not significantly different at alpha = 0.05 level.
[B]Standard deviation.

The mean pseudo-R^2 values were 0.992, 0.996, and 0.996 for the modified Gompertz, Baranyi, and the new models, respectively. The mean BF values were 1.002, 0.999, and 0.999 for the modified Gompertz, Baranyi, and the new models, respectively. The mean AF values were 1.042, 1.023, and 1.034 for the modified Gompertz, Baranyi, and the new models, respectively. Both AF and BF values were not affected by initial inoculum levels and model types used to fit the growth data ($P > 0.27$). The mean RMSE was not influenced by the initial inoculum levels ($P = 0.97$), but it was affected by the model types ($P = 0.001$). At the level of alpha = 0.05, the mean RMSE values were 0.122 and 0.125 (log[CFU/mL]) for the new and Baranyi models, and both were lower than that of the modified Gompertz model (0.193 log[CFU/mL]).

Modeling of isothermal growth in broth—reduced model

Without the data in the stationary phase, the reduced model (Eq. 18) can be directly used to fit the growth curves (Figure 3). Also, as illustrated in Figure 3, the lag phase for each growth curve is visually identifiable and distinguished from the exponential phase. The growth curve in the exponential phase section is clearly linear. Both lag phase and specific growth rate can be directly determined from the new model. The mean lag phase duration determined from the reduced new model was 0.793 h (standard deviation 0.29 h) and was

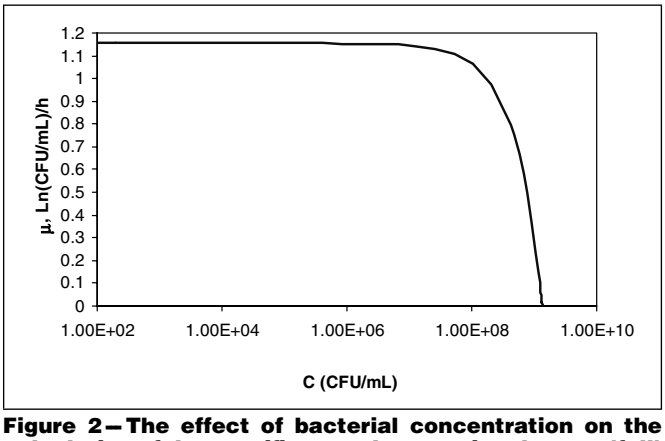

Figure 2—The effect of bacterial concentration on the calculation of the specific growth rate using the new (full) model. This hypothetical curve clearly demonstrates that the bacteria would grow almost at the maximum speed until the cell concentration increases to 1 to 2 logs within the proximity of the maximum cell concentration.

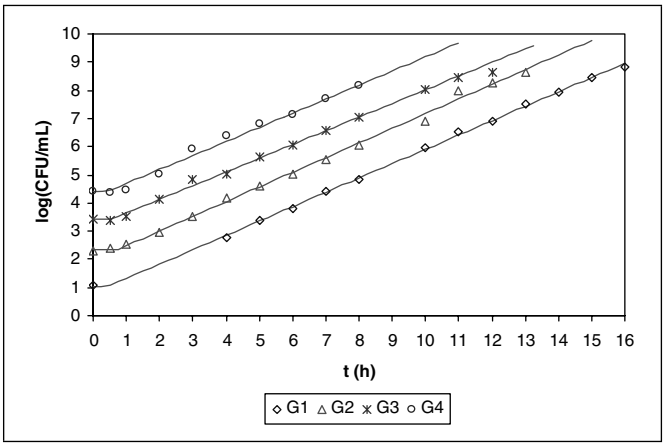

Figure 3—The portions (lag and exponential phases) of the growth of *L. monocytogenes* in TSB modeled by the new (reduced) model.

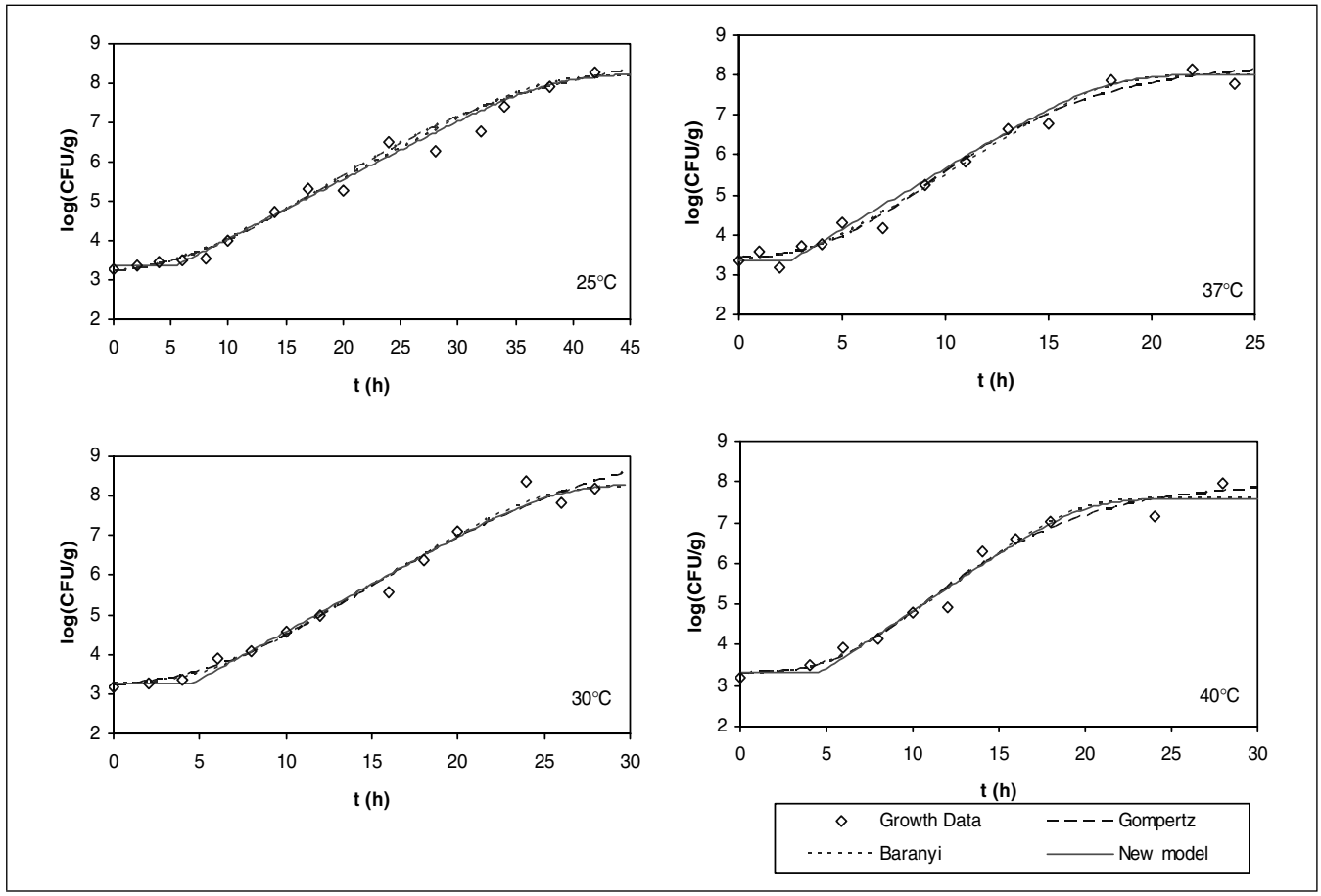

Figure 4—The growth of *L. monocytogenes* in frankfurters modeled by the modified Gompertz, Baranyi, and new (full) models.

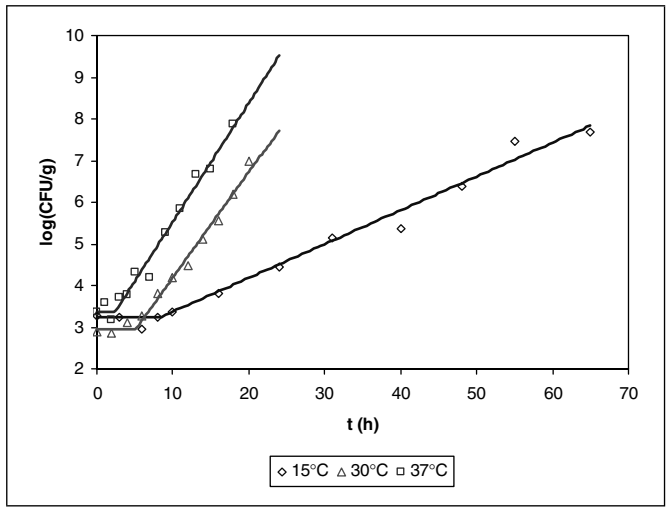

Figure 5—The portions (lag and exponential phases) of the growth of *L. monocytogenes* in frankfurters modeled by the new (reduced) model.

not affected by the initial level of inoculum ($P = 0.524$). The mean specific growth rate determined from the new reduced model was 0.492 log(CFU/mL)/h (standard deviation 0.04), also not affected by the initial level of inoculum ($P = 0.631$). The linear sections of the curves are visibly parallel to each other (Figure 3). The specific growth rate and the duration of the lag phase determined from the reduced model were identical to those determined from the full model.

Modeling of isothermal growth in frankfurters

The growth of *L. monocytogenes* in frankfurters also can be described by the modified Gompertz model, Baranyi model, and the new model (Figure 4). Again, the duration of lag phase of a growth curve, which is affected by temperature, is clearly identifiable by the curves of the new model. For both the modified Gompertz model and the Baranyi model, the microbial growth started gradually from the start of incubation. The duration of the lag phase of the modified Gompertz and the Baranyi models had to be indirectly derived from the curves. Similar to the observations in TSB, the linear nature of the population increase in the exponential phase of bacterial growth was better described by the new model and the Baranyi model. The section of the growth curve corresponding to the exponential phase of the modified Gompertz model was curved. The specific growth rate must be determined from the line tangential to the curve at the inflection point (M) for the modified Gompertz model. Figure 5 shows the effect of temperature on the determination of lag phase duration and specific growth rate from the lag and exponential portions of the growth curves with the new reduced model. The specific growth rates and the lag phase duration were both dependent on temperature ($P < 0.0001$). The choice of a growth model also affected the determination of the specific growth rates and the lag phases of growth curves (Table 2 and 3). In general, there was no significant difference in both specific growth rate (K) and lag phase duration (λ) determined by the new model (full or reduced form) and the Baranyi model (Table 4). The mean specific growth rate determined by the modified Gompertz model was 11% to 12% higher than those determined by the new models (Table 4). Similarly, the mean lag phase duration determined by the modified

Gompertz models was 18% to 20% higher than those determined by the new models (Table 4).

For error analysis, there was no significant difference in the mean values of BF, AF, and RMSE calculated for all temperatures and by all 3 models. The BF values were almost identical to 1. The mean AF values were 1.03. The RMSE values ranged from 0.181 to 0.237 log(CFU/g). All these parameters indicated that all 3 models were equal in accuracy when used to fit the growth data.

Effect of α on K and λ

The success in modeling bacterial growth using the new model, in both full and reduced forms (Eq. 14 and 18), relies on careful selection of the transitional coefficient, α. Figure 6 illustrates the effect of α on the definition of lag phase and exponential phase by the new model (full) for a hypothetical growth curve. In this hypothetical growth curve, the initial and final concentrations are 3 and 9 log (CFU/g); the lag phase duration is 5 h; and the specific growth rate is 0.373 log(CFU/g)/h. Apparently, a small α-value such as $\alpha = 0.1$ does not exhibit either a lag phase or an exponential phase. At $\alpha = 0.5$ or 1.0, the growth curve looks similar to the Baranyi model. As α increases, the transition from the lag phase to the exponential phase becomes more sharply defined, and the lag phase and exponential phase converge smoothly.

Table 2—Mean specific growth rates determined by 3 growth models for growth of *L. monocytogenes* in beef frankfurters.

T (°C)	New model—full	New model—reduced	Baranyi	Modified Gompertz
15	0.090^{c,A} (0.009)^{B}	0.085^{c} (0.005)	0.095^{d} (0.003)	0.100^{d} (0.002)
25	0.181^{b} (0.027)	0.200^{bc} (0.015)	0.188^{c} (0.028)	0.205^{c} (0.030)
30	0.247^{a} (0.007)	0.243^{ab} (0.021)	0.259^{ab} (0.006)	0.266^{b} (0.007)
37	0.289^{a} (0.004)	0.279^{a} (0.011)	0.304^{a} (0.004)	0.330^{a} (0.012)
40	0.277^{a} (0.022)	0.273^{a} (0.027)	0.292^{ab} (0.024)	0.316^{a} (0.022)

[A]Mean. Superscripts with the same letter (lowercase) in the same column were not significantly different at alpha = 0.05 level.
[B]Standard deviation.

Table 3—The mean duration of lag phase determined by 3 growth models for growth of *L. monocytogenes* in beef frankfurters.

T (°C)	New model—full	New model—reduced	Baranyi	Modified Gompertz
15	8.63^{a} (0.09)	8.96^{a} (0.56)	11.28^{a} (1.12)	11.59^{a} (1.08)
25	5.70^{b} (0.60)	6.21^{b} (1.23)	5.57^{b} (0.53)	6.30^{b} (0.54)
30	4.60^{bc} (0.51)	4.14^{bc} (0.83)	4.94^{b} (0.68)	4.85^{bc} (0.78)
37	2.36^{d} (0.30)	2.37^{c} (0.34)	2.65^{c} (0.32)	2.83^{cd} (0.26)
40	3.80^{c} (0.68)	3.81^{c} (0.86)	4.05^{bc} (0.45)	3.45^{d} (0.36)

Superscripts with the same letter in the same column were not significantly different at alpha = 0.05 level.

Table 4—Effect of growth models on the mean specific growth rate and lag phase duration across all temperature conditions for growth of *L. monocytogenes* in beef frankfurters.

Mode	Specific growth rate (per hour)	Lag phase duration (hour)
New model—full	0.217^{b}	5.02^{b}
New model—reduced	0.216^{b}	5.10^{b}
Baranyi	0.228^{ab}	5.70^{ab}
Modified Gompertz	0.243^{a}	6.00^{a}

Superscripts with the same letter in the same column were not significantly different at alpha = 0.05 level.

It would be ideal if α could be directly obtained from nonlinear regression of experimental data to fit Eq. 14 and 18. Since the transitional coefficient α is included in the exponential functions, it is fairly difficult for this number to converge to a definite number without a large number of data points near and around the transitional region of the growth curve. For most growth studies, a small number of data points are obtained. In most cases, the duration of the lag phase of a growth curve is not known beforehand, and therefore it is not practically feasible to collect a large amount of data points in the transitional region. Without sufficient data points in the transitional region, it is difficult to determine the value of α by nonlinear regression.

This technical difficulty can be easily circumvented by treating α as a constant. Theoretically, it is possible to use any number of α values as long as the transition from the lag phase to the exponential phase can be clearly defined. However, it is not practical to use a very large α value since it would cause the curve to change abruptly at the transitional point, which also can cause the divergence of a nonlinear regression process. Based on Figure 6 and preliminary studies, an α value of 25 was chosen for both Eq. 14 and 18. With $\alpha = 25$, nonlinear regression processes will easily converge, and a smooth transition from the lag phase to the exponential phase can be guaranteed. The lag phase and exponential phase can be distinctively defined by the new growth models. In all the nonlinear regression analyses in this study, α was set as 25.

Simplification is not a new model innovation. It was also used to derive the published Baranyi model. According to Baranyi and Roberts (1994), the full Baranyi model is more complex, and is written as

$$y(t) = y_{\max} - \mu_{\max} A(t) - \frac{1}{m} \ln\left[1 + \frac{e^{m\mu_{\max} A(t)} - 1}{e^{m(y_{\max} - y_0)}}\right]$$

where

$$A(t) = t + \frac{1}{\nu} \ln\left(\frac{e^{-\nu t} + q_0}{1 + q_0}\right) \tag{24}$$

The 1st major assumption of this equation is that $m = 1$. Another assumption is that the bacteria are inoculated from a culture in the exponential phase, such as

$$h_0 = \ln\left(1 + \frac{1}{q_0}\right) \tag{25}$$

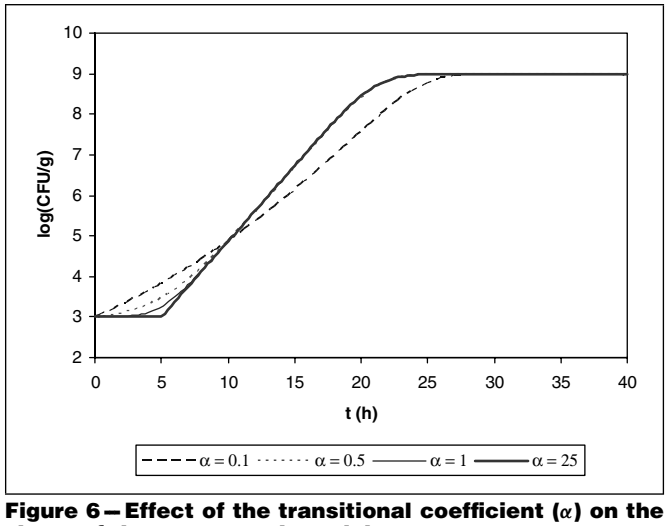

Figure 6—Effect of the transitional coefficient (α) on the shape of the new growth model.

The above-mentioned 2 assumptions reduce the full equation in Eq. 24 to Eq. 4. Another major assumption to make the Baranyi model useful is that $\nu = \mu_{max}$ (Baranyi and others 1995). Without this major assumption, the Baranyi model would not converge during curve fitting. In all the experiments conducted in this study, the stationary phase bacteria culture was used. If the assumption $\nu = \mu_{max}$ was removed, none of the regressions converged.

Conclusions

This study demonstrated that the new mathematical model, both full and reduced forms, could be used to describe the growth of *L. monocytogenes* in broth and in frankfurters. This mathematical model was based on the 3-phase characteristics of bacterial growth under isothermal conditions, and therefore could be considered phenomenologically as a biologically based growth model. Despite the differences in the derivation of all the growth models evaluated in this study, they were equally capable of describing bacterial growth. However, the 3-phase bacterial growth, and particularly the no growth phenomenon in the lag phase and the logarithmic increase in the exponential phase, is more distinct in the new model. Therefore, the model developed in this study, directly derived from the nature of isothermal bacterial growth, may provide advantages over the Baranyi and modified Gompertz models.

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